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Research Article

## Ameliorative effect of *Ipomoea pes-caprae* ethanolic leaf extract on carbohydrate metabolizing enzymes and oxidative status in Streptozotocin-induced diabetic Wistar rats

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### ABSTRACT

In diabetic patients, hyperglycemia is developed due to increased hepatic glucose production and impaired utilization of glucose in cells, which leads to oxidative stress in cells. *Ipomoea pes-caprae* has been widely used as an oral treatment for many diseases. The aim of the present study is to assess the effect of *Ipomoea pes-caprae* ethanolic leaf extract on carbohydrate metabolizing enzymes and oxidative status in Streptozotocin (STZ) induced experimental diabetic rats. Experimental diabetic rats were induced by intraperitoneal administration of 55 mg/kg b.w of STZ. Diabetic rats were treated with *I. pes-caprae* ethanolic leaf extract at a concentration of 300 mg/kg b.w and glibenclamide (6 mg/kg b.w) for 45 days. Diabetic rats exhibited significant ( $P < 0.05$ ) decline in the activity of glucokinase and glucose-6-phosphate dehydrogenase, enzymatic antioxidants (SOD, CAT and GPx) and non-enzymatic antioxidants (GSH, vitamin E and vitamin C), while lipid peroxidation markers (LOOH and TBARS) and glucose-6-phosphatase and fructose-1, 6-bisphosphatase were found to be significantly increased. Further in diabetic rats, the histopathology of pancreas, liver and kidney showed abnormal histo-architecture. The treatment with *Ipomoea pes-caprae* ethanolic leaf extract notably reversed the abnormal levels in carbohydrate metabolizing enzymes, restored the oxidative status and abnormal structures in pancreas, liver and kidney to near normal levels.

**Keywords:** Diabetes, antioxidant, lipid peroxidation, *Ipomoea pes-caprae* and carbohydrate metabolizing enzymes.**Article Info:** Received 07 July 2019; Review Completed 17 August 2019; Accepted 23 August 2019; Available online 30 Aug 2019

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## 1. INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder, is characterized by disruption in the endocrine system due to multiple etiological factors, including chronic hyperglycemia and altered carbohydrate, fats, and proteins metabolism, which is caused by the defects in insulin secretion and/or action [1]. The prevalence of DM is expected to further increase to 9.9% (95% CI 7.5-12.7%) by the year 2045. In total, this reflects a population of 424.9 million people with diabetes worldwide in 2017 with an estimate of a 48% increase to 628.6 million people (95% CI 477.0-808.7 million) for the year 2045 [2].

The chronic hyperglycemia may lead to long-term oxidative cellular injury and dysfunction of various organs, particularly the kidneys, eyes, nerves, liver, heart, and blood vessels. The provoked oxidative stress and lipid

peroxidation act as a potential pathophysiological mechanism underlying diabetic complications through the overproduction of reactive oxygen species and by triggering the production of proinflammatory cytokines, which together with reactive oxygen species are deemed to play vital roles in the pathogenesis of DM [3].

Nowadays, the utilization of herbal derived drugs has increased because of their superior activity, lesser side effects, as well as relatively low production cost. Thus, screening of novel antidiabetic alternative drugs from natural plant sources is of profound importance for the world [4].

*I. pes-caprae* is a common pan-tropical creeping vine belonging to the family Convolvulaceae. This plant is used as a medicinal plant in many countries for the treatment of several ailments, including anti-inflammatory and analgesic

treatments [5]. All parts of *I. pes-caprae* are medicinally useful and are used in folk and traditional medicines for treating different diseases like stomach pain, fever and rheumatoid arthritis, etc., [6]. Earlier, some researchers reported that *I. pes-caprae* shows many biological activities such as anti-inflammatory activity Venkataraman et al. [7], anticancer activity Robert and Retna [8], antibacterial and antifungal activities (Bragadeeswaran et al. [9] etc. Earlier, we reported that ethanolic leaf extract of *I. pes-caprae* regulated the abnormal blood glucose level and hepatic markers in STZ induced diabetic rats (Suhasini et al. [10]. The objective of this present study is to observe the effect of ethanolic leaf extract of *I. pes-caprae* on carbohydrate metabolizing enzymes, oxidative status and major organs (pancreas, liver and kidney) in STZ induced diabetic rats.

## 2. MATERIALS AND METHODS

### 2.1. Experimental animals

Male Wistar rats weighing 150-200 gms were used for the present study. The animals were obtained from the central animal house, Rajah Muthiah Institute of Health Sciences, Annamalai University, India and were maintained at 12h light: dark cycles. All the animals were randomly divided into experimental and control groups. Animals were housed as 5 or 6 in each polypropylene cage. Standard pellets were obtained from Mysore snack feed Ltd., Mysore, was used as a basal diet during the entire experimental period. The control and the experimental animals were provided with food and drinking water *ad libitum*.

### 2.2. Chemicals

STZ was purchased from Sigma- Aldrich Pvt. Ltd., Bangalore, India. All the other chemicals as well as reagents used in this experiment were of analytical grade.

### 2.3. Preparation of ethanolic leaf extract of *Ipomoea pes-caprae*

*I. pes-caprae* was collected from Parangipettai, Tamil Nadu, India, and was identified by the Department of Botany, Annamalai University. The *Ipomoea pes-caprae* (Convolvulaceae) leaves were then separated and dried at room temperature, under the shade, and was powdered using a dry grinder and then passed through a sieve. Dried leaf powder (100 grams) of *I. pes-caprae* was packed in a Soxhlet apparatus and was extracted with ethanol. The ethanol extract obtained was concentrated using a rotary evaporator.

### 2.4. Induction of diabetes

Diabetes was induced in male albino Wistar rats by a single intraperitoneal (I.P.) injection of streptozotocin (STZ) at a dose of (55 mg/kg b.w) dissolved in 0.1 M cold citrate buffer (pH = 4.5). The rats were then allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. When the fasting blood glucose values reached above 250 mg/dl, on the third day after STZ injection, then the rats were considered as diabetic.

### 2.5. Experimental design

The animals were maintained in accordance with the "CPCSEA guidelines for laboratory animal facility" (CPCSEA: Committee for the purpose of control and supervision of experiments on animals) and the approval number is (Pro.No.1030; Reg No.160/1999/CPCSE). The treatment of ethanolic leaf extract of *I. pes-caprae* at concentration of 300 mg/kg b.w (dissolved in 0.05% dimethyl sulfoxide (DMSO)) and glibenclamide (reference drug) at concentration of 6 mg/kg b.w was started on the fifth day after STZ injection

and it was considered as the first day of treatment. Treatment was given for 45 days orally whereas control rats were administered with drug vehicle, i.e., 0.05% DMSO, and diabetic rats were fed with water alone.

A total number of 24 rats were used and the rats were divided into 4 groups of 6 animals in each group.

Group 1 : Control rats (drug vehicle: 0.05% DMSO)

Group 2 : Diabetic control rats administered intraperitoneally with streptozotocin (as single dose: 55 mg/kg b.w).

Group 3 : Diabetic rats administered with ethanolic extract of *Ipomoea pes-caprae* leaf (300 mg/kg of body weight) for a period of 45 days.

Group 4 : Diabetic rats administered with reference drug glibenclamide (6 mg/kg of body weight) for a period of 45 days.

On the 45th day, all the animals were sacrificed by cervical dislocation, and the blood samples were collected for analysis of biochemical parameters. The pancreas, liver and kidney were excised and washed with ice-cold saline to observe levels of carbohydrate metabolizing enzymes, antioxidant status and histopathological analysis.

### 2.6. Determination of carbohydrate metabolizing enzymes

Liver glycogen was evaluated by the method of Shirwaikar et al. [11]. The activity of hepatic glucokinase by the method of Brandstrup et al. [12], glucose-6-phosphate dehydrogenase by the method of Ellis and Kirkman [13], glucose-6-phosphatase by the method of Koide and Oda [14] and fructose-1, 6-bisphosphatase activity were estimated by the method of Gancedo and Gancedo [15].

### 2.7. Estimation of lipid peroxidation and antioxidants

The activity of the thiobarbituric acid reactive substance (TBARS) and lipid peroxide (LOOH) in the liver was measured by the method of Ohkawa [16]. The activity of superoxide dismutase (SOD) was evaluated by the method of Kakkar [17]. The activity of catalase (CAT) enzyme was measured by the method given by Sinha, [18]. Glutathione Peroxidase (GPx) was evaluated by the method of Rotruck [19]. The activity of reduced glutathione (GSH) was assessed by the method of Ellman, [20]. Vitamin C and E levels were evaluated by the method of Omaye et al. [21] and Baker and Frank [22], respectively.

### 2.8. Histopathology

The harvested organs (pancreas, liver and kidney) were fixed in 10 % formalin for 48hrs. It was then followed by dehydration by passing through a series of graded alcohol and were finally embedded in paraffin blocks. Sections of pancreas, liver and kidney (5-6  $\mu$ m thick) were developed using semi-automated rotator microtome. Finally, the sections obtained were stained with Hematoxylin and Eosin (H & E) dye on a glass slide and observed microscopically.

### 2.9. Statistical analysis

All biochemical data were expressed as mean  $\pm$  SEM. All the statistical analysis was performed using the One-way ANOVA followed by Tukey's multiple tests using SPSS (version 18) of computer software. In all the cases, a p value of less than 0.05 was considered to be statistically significant.

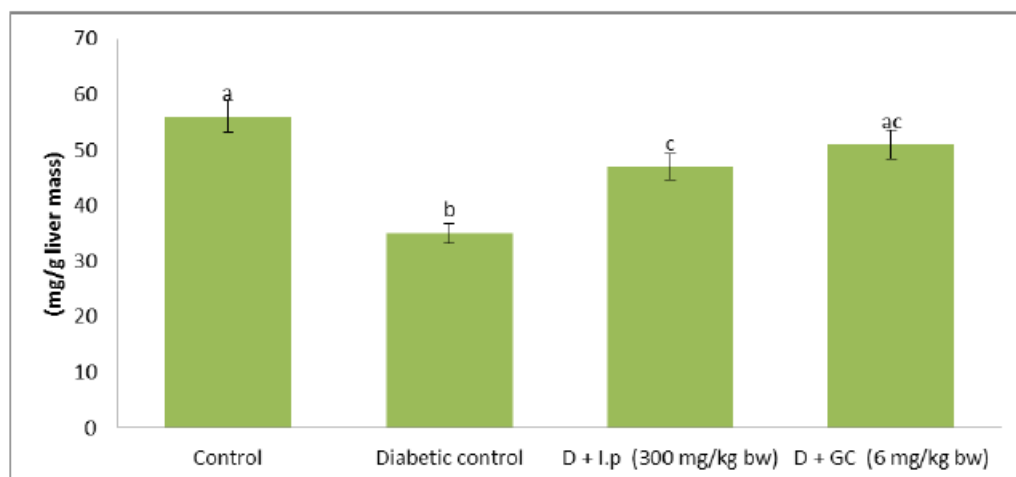
### 3. RESULTS

#### 3.1. Effect of *Ipomoea pes-caprae* on liver glycogen

Figure 1 shows the level of liver glycogen in normal and experimental rats. The level of liver glycogen was

significantly reduced in diabetic control rats when compared to control rats. Treatment with *I. pes-caprae* leaf ethanolic extract and glibenclamide significantly improved the glycogen levels in the liver of diabetic rats when compared to diabetic control rats.

**Figure 1: The liver glycogen levels in experimental and control rats**



All the data were expressed as the mean  $\pm$  S.D. for 6 rats. The results with different superscripts (a,b,c..) in each experimental groups are significantly different at  $p < 0.05$ .

(D: Diabetic; I.p: *I. pes-caprae* leaf ethanolic extract; GC: Glibenclamide)

#### 3.2. Effect of *Ipomoea pes-caprae* on carbohydrate metabolizing enzymes

Table 1 shows the level of carbohydrate metabolizing enzymes in experimental and control rats. The activity of hepatic glucokinase and glucose-6-phosphate

dehydrogenase significantly declined, whereas glucose-6-phosphatase and fructose-1, 6-bisphosphatase activity significantly elevated in the diabetic control rats. The abnormal levels of carbohydrate metabolizing enzymes were improved by *I. pes-caprae* leaf ethanol extract and glibenclamide treatment.

**Table 1: The liver carbohydrate metabolizing enzyme levels in experimental and control rats**

Groups	Hexokinase (U*/h/ mg protein)	Glucose-6-phosphate dehydrogenase (U#/mg protein)	Glucose-6 phosphatase (Unit@/min/ mg protein)	Fructose 1,6-bis phosphatase (Unit\$/h/ mg protein)
Control	0.30 $\pm$ 0.03 <sup>a</sup>	4.62 $\pm$ 0.36 <sup>a</sup>	5.76 $\pm$ 0.41 <sup>a</sup>	13.41 $\pm$ 0.99 <sup>a</sup>
Diabetic control	0.10 $\pm$ 0.01 <sup>b</sup>	2.57 $\pm$ 0.19 <sup>b</sup>	7.72 $\pm$ 0.61 <sup>b</sup>	26.47 $\pm$ 2.05 <sup>b</sup>
D + I.p (300 mg/kg bw)	0.25 $\pm$ 0.03 <sup>c</sup>	3.79 $\pm$ 0.29 <sup>c</sup>	6.15 $\pm$ 0.47 <sup>c</sup>	16.44 $\pm$ 1.26 <sup>c</sup>
D + GC (6 mg/kg bw)	0.28 $\pm$ 0.03 <sup>ac</sup>	4.18 $\pm$ 0.42 <sup>ac</sup>	5.47 $\pm$ 0.42 <sup>ac</sup>	13.76 $\pm$ 1.01 <sup>a</sup>

All the data are expressed as the mean  $\pm$  S.D. for 6 rats. The results with different superscripts (a,b,c..) in each experimental groups are significantly different at  $p < 0.05$ .

(D: Diabetic; I.p: *I. pes-caprae* leaf ethanolic extract; GC: Glibenclamide)

#### 3.3. Effect of *Ipomoea pes-caprae* on lipid peroxidation

TBARS and LOOH levels were significantly ( $p < 0.05$ ) elevated in the organs (pancreas, liver and kidney) of diabetic control rats when compared to control rats,

whereas diabetic rats treated with *I. pes-caprae* leaf ethanol extract and glibenclamide significantly inhibited the increased level of TBARS and LOOH as compared with diabetic control rats (Table 2).

**Table 2: The levels of lipid peroxidation markers (TBARS and LOOH) in different tissues of experimental and control rats**

Groups	Control	Diabetic control	D + <i>Lp</i> (300 mg)	D + GC (6 mg)
<b>TBARS<sup>1</sup></b>				
Liver	0.79 ± 0.06 <sup>a</sup>	3.09 ± 0.20 <sup>b</sup>	1.77 ± 0.14 <sup>c</sup>	1.47 ± 0.12 <sup>c</sup>
Kidney	1.32 ± 0.11 <sup>a</sup>	3.32 ± 0.23 <sup>b</sup>	2.19 ± 0.15 <sup>c</sup>	1.63 ± 0.13 <sup>a</sup>
Pancreas	0.30 ± 0.03 <sup>a</sup>	0.49 ± 0.03 <sup>b</sup>	0.38 ± 0.03 <sup>c</sup>	0.37 ± 0.03 <sup>a</sup>
<b>LOOH<sup>2</sup></b>				
Liver	72.10 ± 5.54 <sup>a</sup>	124.62 ± 9.66 <sup>b</sup>	84.78 ± 6.61 <sup>c</sup>	77.30 ± 5.72 <sup>ac</sup>
Kidney	66.77 ± 5.10 <sup>a</sup>	153.17 ± 11.89 <sup>b</sup>	78.68 ± 6.01 <sup>c</sup>	75.18 ± 5.24 <sup>ac</sup>
Pancreas	16.54 ± 1.27 <sup>a</sup>	29.38 ± 2.25 <sup>b</sup>	18.63 ± 1.61 <sup>c</sup>	17.27 ± 1.27 <sup>ac</sup>

<sup>1</sup>TBARS in tissues were expressed as μmoles/g tissue

<sup>2</sup>LOOH in tissues were expressed as x10<sup>-5</sup> mmoles/g tissue

All the data are expressed as the mean ± S.D. for 6 rats. The results with different superscripts (a,b,c..) in each experimental group are significantly different at  $p < 0.05$ .

(D: Diabetic; Lp: *I. pes-caprae* leaf ethanolic extract; GC: Glibenclamide)

### 3.4. Effect of *Ipomoea pes-caprae* on enzymatic antioxidant enzymes

Table 3 depicts the enzymatic antioxidant activities in organs (pancreas, liver and kidney) of experimental and control rats. The activity of enzymatic antioxidant enzymes

such as SOD, CAT and GPx were significantly declined in diabetes control rats when compared with control rats. *I. pes-caprae* leaf ethanol extract and glibenclamide treated diabetic rats showed significant improvement in the activity of SOD, CAT and GPx as compared to diabetic control rats.

**Table 3: The levels of enzymatic antioxidants in different tissues of experimental and control rats**

Groups	Control	Diabetic control	D + BC (50 mg)	D + GC (6 mg)
<b>SOD<sup>1</sup></b>				
Liver	9.34 ± 0.71 <sup>a</sup>	4.78 ± 0.34 <sup>b</sup>	7.76 ± 0.62 <sup>c</sup>	8.39 ± 0.67 <sup>a</sup>
Kidney	14.31 ± 1.10 <sup>a</sup>	6.49 ± 0.52 <sup>b</sup>	12.34 ± 0.93 <sup>c</sup>	12.64 ± 1.01 <sup>ac</sup>
Pancreas	3.19 ± 0.24 <sup>a</sup>	1.67 ± 0.12 <sup>b</sup>	2.73 ± 0.20 <sup>c</sup>	2.82 ± 0.21 <sup>a</sup>
<b>CAT<sup>2</sup></b>				
Liver	80.06 ± 6.17 <sup>a</sup>	51.67 ± 4.03 <sup>b</sup>	67.78 ± 5.13 <sup>c</sup>	70.35 ± 5.29 <sup>ac</sup>
Kidney	40.23 ± 3.07 <sup>a</sup>	18.21 ± 1.38 <sup>b</sup>	32.58 ± 2.51 <sup>c</sup>	35.14 ± 2.71 <sup>a</sup>
Pancreas	0.74 ± 0.05 <sup>a</sup>	0.39 ± 0.03 <sup>b</sup>	0.64 ± 0.05 <sup>c</sup>	0.72 ± 0.05 <sup>a</sup>
<b>GPx<sup>3</sup></b>				
Liver	11.21 ± 0.87 <sup>a</sup>	4.82 ± 0.37 <sup>b</sup>	9.20 ± 0.71 <sup>c</sup>	10.38 ± 0.79 <sup>ac</sup>
Kidney	8.38 ± 0.66 <sup>a</sup>	4.26 ± 0.31 <sup>b</sup>	6.59 ± 0.53 <sup>c</sup>	7.64 ± 0.58 <sup>a</sup>
Pancreas	1.69 ± 0.12 <sup>a</sup>	0.97 ± 0.08 <sup>b</sup>	1.38 ± 0.11 <sup>c</sup>	1.59 ± 0.12 <sup>ac</sup>

<sup>1</sup>SOD for tissues were expressed as 50% inhibition of nitroblue tetrazolium reduced in 1minute/mg protein

<sup>2</sup>CAT for tissues were expressed as μmoles of H<sub>2</sub>O<sub>2</sub> consumed/ minute/mg protein.

<sup>3</sup>GPx for tissues were expressed as μg of GSH consumed /minute/mg protein

All the data are expressed as the mean ± S.D. for 6 rats. The results with different superscripts (a, b, c..) in each experimental groups are significantly different at  $p < 0.05$ .

(D: Diabetic; Lp: *I. pes-caprae* leaf ethanolic extract; GC: Glibenclamide)

### 3.5. Effect of *Ipomoea pes-caprae* on non-enzymatic antioxidants

Table 4 shows the level of non-enzymatic antioxidants in tissue (pancreas, liver and kidney) of control and experimental rats. The GSH, vitamin C and E levels were

significantly declined in diabetic control rats when compared to control rats. These abnormal levels of non-enzymatic antioxidants improved considerably to near normal levels in *I. pes-caprae* leaf ethanol extract and glibenclamide treated diabetic rats.



**Table 4: The levels of non-enzymatic antioxidants in different tissues of experimental and control rats**

Groups	Control	Diabetic control	D + I. p (300 mg/kg bw)	D + GC (6 mg/kg bw)
<b>GSH<sup>1</sup></b>				
Plasma	30.19 ± 2.39 <sup>a</sup>	15.67 ± 1.17 <sup>b</sup>	24.18 ± 1.79 <sup>c</sup>	25.09 ± 1.89 <sup>c</sup>
Liver	13.36 ± 1.09 <sup>a</sup>	8.07 ± 0.61 <sup>b</sup>	11.79 ± 0.89 <sup>c</sup>	12.88 ± 0.96 <sup>ac</sup>
Kidney	12.49 ± 0.96 <sup>a</sup>	6.61 ± 0.50 <sup>b</sup>	10.28 ± 0.80 <sup>c</sup>	11.29 ± 0.86 <sup>ac</sup>
Heart	10.21 ± 0.78 <sup>a</sup>	5.25 ± 0.41 <sup>b</sup>	8.75 ± 0.68 <sup>c</sup>	9.10 ± 0.70 <sup>ac</sup>
Pancreas	1.78 ± 0.13 <sup>a</sup>	0.90 ± 0.07 <sup>b</sup>	1.39 ± 0.11 <sup>c</sup>	1.55 ± 0.12 <sup>a</sup>
<b>Vitamin C<sup>2</sup></b>				
Plasma	2.19 ± 0.11 <sup>a</sup>	0.75 ± 0.09 <sup>b</sup>	1.64 ± 0.13 <sup>c</sup>	1.89 ± 0.14 <sup>ac</sup>
Liver	0.89 ± 0.08 <sup>a</sup>	0.44 ± 0.03 <sup>b</sup>	0.72 ± 0.06 <sup>c</sup>	0.83 ± 0.08 <sup>a</sup>
Kidney	0.79 ± 0.06 <sup>a</sup>	0.53 ± 0.05 <sup>b</sup>	0.69 ± 0.05 <sup>c</sup>	0.76 ± 0.06 <sup>ac</sup>
Heart	0.49 ± 0.03 <sup>a</sup>	0.23 ± 0.02 <sup>b</sup>	0.39 ± 0.03 <sup>c</sup>	0.47 ± 0.04 <sup>ac</sup>
Pancreas	2.41 ± 0.18 <sup>a</sup>	1.32 ± 0.10 <sup>b</sup>	2.09 ± 0.16 <sup>c</sup>	2.30 ± 0.18 <sup>ac</sup>
<b>Vitamin E<sup>2</sup></b>				
Plasma	1.89 ± 0.14 <sup>a</sup>	3.21 ± 0.27 <sup>b</sup>	2.32 ± 0.19 <sup>c</sup>	2.17 ± 0.18 <sup>c</sup>
Liver	5.79 ± 0.46 <sup>a</sup>	3.88 ± 0.29 <sup>b</sup>	4.48 ± 0.33 <sup>c</sup>	4.99 ± 0.39 <sup>c</sup>
Kidney	3.59 ± 0.27 <sup>a</sup>	1.30 ± 0.10 <sup>b</sup>	2.89 ± 0.23 <sup>c</sup>	3.02 ± 0.29 <sup>a</sup>
Heart	3.44 ± 0.25 <sup>a</sup>	1.63 ± 0.13 <sup>b</sup>	2.84 ± 0.21 <sup>c</sup>	3.05 ± 0.23 <sup>a</sup>
Pancreas	2.69 ± 0.21 <sup>a</sup>	1.37 ± 0.11 <sup>b</sup>	2.51 ± 0.19 <sup>c</sup>	2.77 ± 0.25 <sup>ac</sup>

<sup>1</sup>GSH for tissues were expressed as µg/mg protein

<sup>2</sup>Vitamin E and vitamin C in plasma and tissues were expressed as µmole/mg tissue respectively.

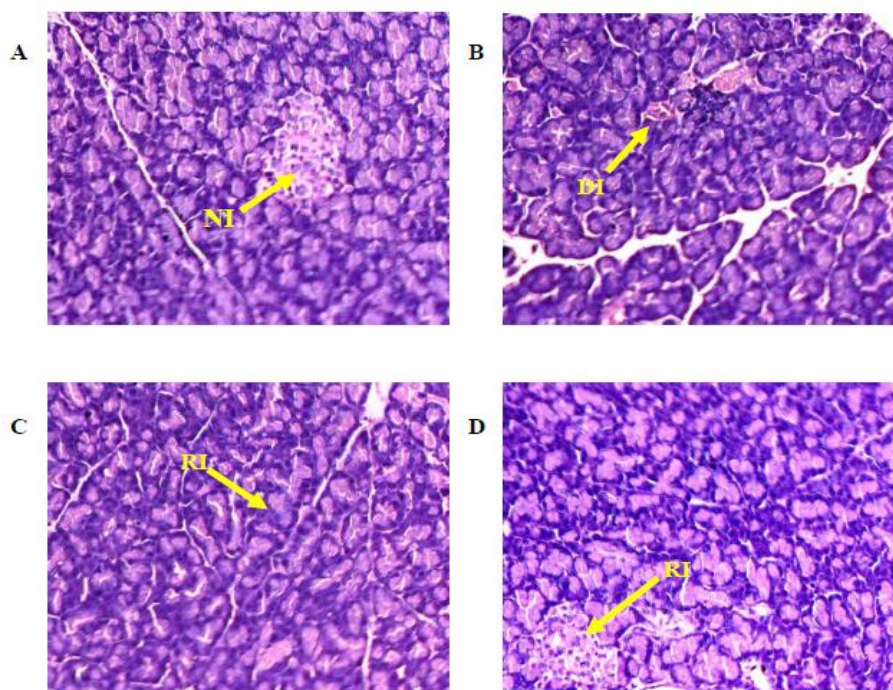
All the data are expressed as the mean ± S.D. for 6 rats. The results with different superscripts (a, b, c..) in each experimental group are significantly different at  $p < 0.05$ .

(D: Diabetic; I.p: *I. pes-caprae* leaf ethanolic extract; GC: Glibenclamide)

### 3.6. Histopathology of Pancreas

Figure 2 illustrates the histology of experimental and control rats. The pancreas, the target tissue of diabetic etiology, showed severe damage in the case of STZ treated animals. The sinusoids as well as the islet cells of the control animals

displayed normal tissue architecture, whereas, the STZ treated animals showed necrotic cells in the tissue sections. *I. pes-caprae* extract administered groups showed regeneration of islet cells and the same effect were observed with standard glibenclamide treated group.

**Figure 2: Histopathology of pancreas in experimental and control rats**

A- Control Pancreas (NI: Normal Islets); B- Diabetic Pancreas (DI: Damaged Islets)

C- *Ipomoea pes-caprae* extract treated Pancreas (RI: Recovering Islets)

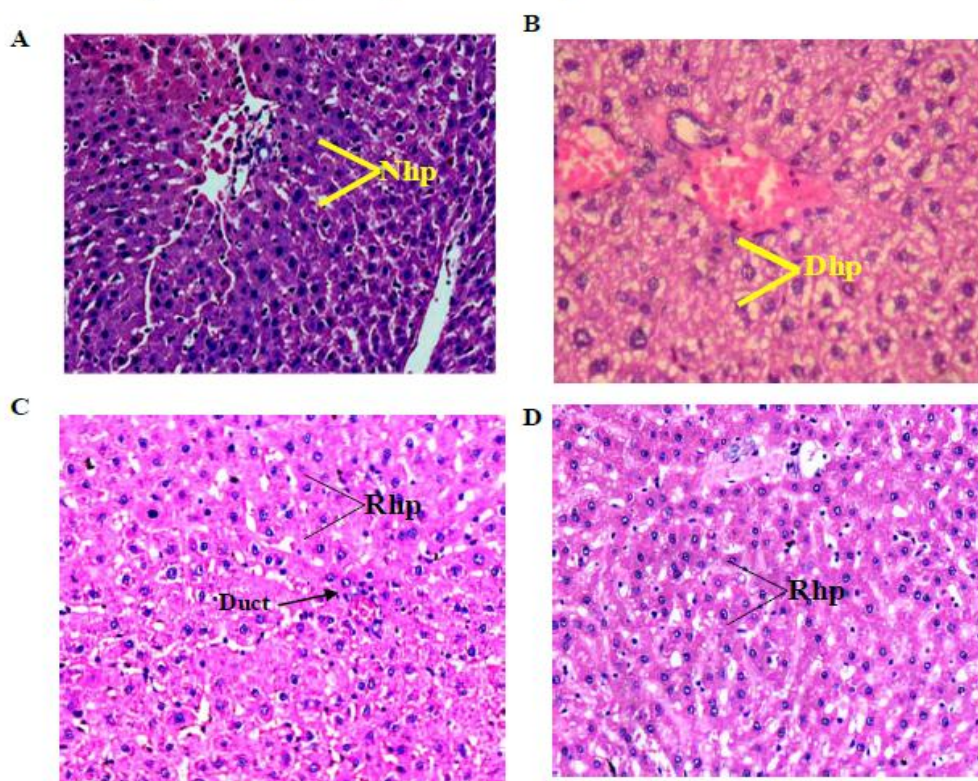
D- Glibenclamide treated Pancreas (RI: Recovered Islets)

### 3.7. Histopathology of Liver

The histopathology of liver tissues of control animals revealed ideal hepatocytes whereas, STZ treated animals displayed distorted tissue architecture and damaged liver hepatocytes accompanied with degeneration in the major

blood sinusoids were undergoing degeneration, which are the characteristics of STZ induced diabetic animal liver. The *I. pes-caprae* extract and glibenclamide drug-treated groups showed tissue regeneration with prominent near normal hepatocytes (Figure 3).

**Figure 3: Histopathology of liver in experimental and control rats**



**A- Control liver (Nhp: Normal hepatocytes)**

**B- Diabetic liver (STZ treated) (Dhp: Damaged hepatocytes)**

**C- *Ipomoea pes-caprae* extract treated liver (Rhp: Recovered hepatocytes)**

**D- Glibenclamide treated liver (Standard Drug)**

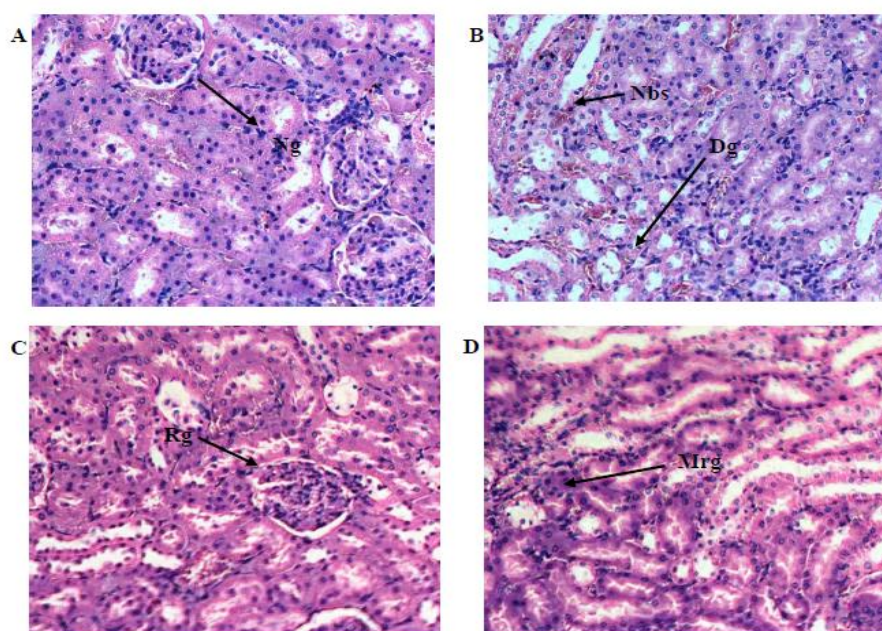
### 3.8. Histopathology of Kidney

The kidney, the ultrafiltration unit of the body, plays a major role in glucose absorption. STZ administration causes severe necrosis accompanied by glomeruli damage in the kidney of

diabetic control rat. *Ipomoea pes-caprae* extract treated diabetic animals displayed considerable tissue recovery and the results were comparable to glibenclamide (standard drug) treated animals (Figure 4).



Figure 4: histopathology of kidney in experimental and control rats



A- Control kidney (Normal treated) (Ng: Normal glomeruli)

B- Diabetic kidney (Nbs: Necrosized sinusoids; Dg: Damaged glomeruli)

C- *Ipomoea pes-caprae* extract treated kidney (Rg: Regenerative glomeruli)

D- Glibenclamide treated kidney (Mrg: Moderate regenerative glomeruli)

## DISCUSSION

Medicinal plants have created the foundation of the health care system throughout the world since the initial stage of humanity and still plant products is the major source of drug/formulation in the treatment of various diseases [4]. Previously, we have confirmed that *Ipomoea pes-caprae* leaf ethanolic extract can reduce hyperglycemia and relapse abnormal level of hepatic markers into normal range in STZ induced diabetic rats [10]. Therefore, the goal of this study is to observe whether *Ipomoea pes-caprae* leaf ethanolic extract ameliorated the abnormal levels of carbohydrate metabolizing enzymes, oxidative status and histology of major organs in STZ induced diabetic rats.

Glycogen is the primary intracellular storage form of glucose and its quantity in various tissues is a direct manifestation of insulin activity as insulin supports intracellular glycogen deposition Pederson et al. [23]. The reduced glycogen store in diabetic rats has been attributed to the loss of glycogen synthase-activating system and/or the increased activity of glycogen phosphorylase [24]. In the present study, there was a decrease in the hepatic glycogen content of diabetic rats that suggests the increased glucose output due to insulin deficiency. Moreover, diabetic animals showed increased glycogen phosphorylase activity when compared with control animals. Treatment with *Ipomoea pes-caprae* ethanolic leaf extract restored the levels of glycogen, probably by means of decreasing the activity of glycogen phosphorylase.

Glucose homeostasis deals with the balance in the endogenous production as well as the glucose utilization by the target tissues [25]. Insulin, a major player in glucose homeostasis, is responsible for regulating the activities of various enzymes like hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase as well as fructose

1,6-bisphosphatase, which are associated with carbohydrate metabolism in the body [26]. Glucokinase, a key enzyme involved in glycolysis, catalyzes the conversion of glucose to glucose 6-phosphate (in the liver) and plays a very important role in maintaining blood glucose homeostasis [27]. Being an insulin-dependent enzyme, the hepatic glucokinase activity of diabetic rats is almost entirely inhibited or inactivated due to the lack of insulin that ultimately leads to hyperglycaemia [27]. The diabetic control rats showed a significant reduction in the level of hexokinase when compared to control rats. Oral administration of *I. pes-caprae* ethanol leaf extract to STZ-induced diabetic rats resulted in a significant reversal in the activity of glucokinase, thereby increasing the oxidation of glucose. Several previous studies reported that glucokinase is the potential target for new treatment strategies for the management of type 2 diabetes [24, 26].

Glucose-6-phosphate dehydrogenase is the first and also the rate-limiting enzyme of the pentose phosphate pathway, which is involved in the formation of ribose-5-phosphate and also NADPH [29]. Thus, its low activity leads to a decrease in NADPH and makes cells very sensitive to oxidative damage. In the diabetic condition, the decrease in the activity of glucose-6-phosphate dehydrogenase hinders the pentose phosphate pathway [32]. In the present study, treatment with *I. pes-caprae* ethanolic leaf extract improved the activity of glucose-6-phosphate dehydrogenase in STZ-induced diabetic rats that may be due to the improved level of insulin secretion.

Glucose-6-phosphatase and fructose-1, 6-bisphosphatase are the regulatory enzymes in the gluconeogenic pathway. The actions of these two enzymes may be attributed to the increased glucose production during the diabetic condition by the liver [30]. The activities of hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase increase

significantly in diabetic rats [31]. The oral treatment of *I. pes-caprae* ethanolic leaf extract significantly declined the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in STZ-induced diabetic rats, which may lead to the suppression of glucose production from non-carbohydrate substances.

In the present study, the concentration of tissue TBARS was significantly increased in the liver and kidney and pancreas of diabetic rats as reported earlier in clinical and experimental diabetes [32]. The increased concentration of lipid peroxidative markers suggests an increased level of oxygen free radicals, either by increased production or decreased destruction [33]. The administration of *Ipomoea pes-caprae* leaf extract reduced TBARS in the tissues of diabetic rats suggesting that the extract possesses potent antioxidant properties. The strong antioxidant property displayed by the extract is in accordance with earlier findings [34].

The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic scavenger systems [35]. SOD, CAT and GPx constitute a mutually supportive defense system against ROS. Functions of these antioxidant enzymes are interconnected and a decrease of their activities results in the accumulation of lipid peroxides and increase oxidative stress in diabetic rats [36]. The level of enzymatic antioxidants (SOD, CAT and GPx) was significantly restored to near normal after treatment with *Ipomoea pes-caprae* leaf extract in diabetic rats. This indicates the modulatory activity of *Ipomoea pes-caprae* leaf extract over SOD, CAT and GPx enzyme production, which help restore the oxidant status in diabetic rats.

Glutathione is a tripeptide, intracellular non-enzymic antioxidant that protects the cellular system from adverse effects of lipid peroxidation. It directly scavenges free radicals and acts as a co-substrate for peroxide detoxification by glutathione peroxidases [37]. Increased oxidative stress due to a significant increase in aldehydic products of lipid peroxidation could probably decrease GSH availability in the cell [38]. It has been previously reported that the treatment with *Ipomoea pes-caprae* leaf extract result in the elevation of the GSH levels, which protects the cell membrane from the oxidative damage by regulating the redox status [39]. Vitamin C plays a vital role in the antioxidant system that protects all lipids from undergoing oxidation. Moreover, it helps in diminishing count of apoptotic cells [40] and helps to regenerate vitamin E from its oxidized state [41]. Vitamin E is a non-enzymatic antioxidant that helps reduce chain reactions associated with lipid peroxidation [42]. Vitamin E is also reported to be very effective in the glycemic control and also helps in lowering the level of HbA1c, the glycated haemoglobin, used as a marker to measure average blood glucose [43]. In our studies, diabetic control rats showed significant decline in the levels of GSH, vitamin C and vitamin E, while *Ipomoea pes-caprae* leaf extract administration in STZ-induced experimental DM rats expressed near normal levels of GSH, vitamin C and E. Thus, the obtained results display the potential of *Ipomoea pes-caprae* ethanolic leaf extract to restore the non-enzymatic and enzymatic antioxidant reserves.

The most abundant cell type found in the endocrine region of the pancreas is the  $\beta$ -cell. The number of  $\beta$ -cell present in the pancreas is the most vital factor determinant of the islet area [32]. The pancreas of the diabetic rats displayed ruptured islets as well as a decrease in the number of  $\beta$ -cell count. Thus, the islet area was reduced considerably in the

diabetic rats, which was found restored in *Ipomoea pes-caprae* extract and glibenclamide treated diabetic rats. The restoration of damages in the pancreas may be due to the improved control over the activity of free radical by the *Ipomoea pes-caprae* extract.

The liver is the vital organ that is involved in the blood glucose homeostasis in the body. Both the liver and kidney exhibit various morphological as well as functional modifications during diabetes [31]. The diabetic control liver showed damaged hepatocytes with periportal necrosis near the portal region with dilated as well as congestion in the portal vessels and inflammatory cell infiltration. Moreover, the diabetic kidney displayed damaged tubules, lesions and fatty infiltration along with thickened glomeruli matrix and basement membranes of proximal as well as distal convoluted tubules. The above observations in diabetic control liver and kidney tissues were in line with the previous reportings by Ojiako et al. [44]. Hyperglycemias as well as glycation of proteins are the main cause of the damages that occur in diabetic rats [44]. The restoration of damages in the liver and kidney of diabetic rats treated with *Ipomoea pes-caprae* extract and glibenclamide may be due to improved glycemic control and thereby control over free radical production and glycation of proteins.

The effectiveness of the *Ipomoea pes-caprae* leaf extract in ameliorating the glucose and oxidant status in the diabetic rats may be contributed to the presence of secondary metabolites such phenols, sterols, alkaloids, flavonoids, etc., in leaf of *Ipomoea pes-caprae* [45, 46].

## CONCLUSION

The treatment of *Ipomoea pes-caprae* (300 mg/kg b.w) notably inhibits the oxidative stress, while carbohydrate metabolizing enzyme levels were also improved in the liver of diabetic rats. These findings clearly displayed recovered carbohydrate metabolizing enzymes that prevent hepatic glucose production and also enhanced utilization of glucose by cells. Further study is required to find out the major secondary metabolites that are responsible for regulation of hyperglycemia in diabetic condition.

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